Out-of-equilibrium collective oscillation as phonon condensation in a model protein

Ilaria Nardecchia,[1,2,3] Jeremie Torres,[4] Mathias Lechelon,[1,2,3] Valeria Giliberti,[5] Michele Ortolani,[5] Philippe Nouvel,[4] Matteo Gori,[1,2] Irene Donato,[1,2] Jordane Preto,[6] Luca Varani,[4] James Sturgis,[7] Marco Pettini[1,2*]

[1]Aix-Marseille University, Marseille, France,
[2]CNRS Centre de Physique Théorique UMR7332, 13288 Marseille, France
[3]Centre d'Immunologie de Marseille-Luminy, 13288 Marseille, France,
[4]IES, University of Montpellier – CNRS, UMR 5214, 34000 Montpellier, France,
[5]Department of Physics, University of Rome "La Sapienza", Italy,
[6]Rice University, Houston, USA,
[7]Laboratoire d'Ingénierie des Systèmes Macromoléculaires UMR7255, 13402 Marseille, France,

*To whom correspondence should be addressed; E-mail: pettini@cpt.univ-mrs.fr.

A longstanding (*1, 2*), and still present (*3–5*), proposal relates the activation of collective intra-molecular oscillations of biomolecules with their biological functioning. These collective oscillations have been hitherto studied only at thermal equilibrium. On the basis of a theoretical model, inspired by a longstanding proposal, a Bose-like phenomenon of phonon (as protein-normal modes) condensation is predicted to occur out-of-thermal-equilibrium, that is, through an energy flow due to the combined effects of energy pumping and dissipation. This is expected to bring about giant oscillating molecular dipole moments, a necessary condition to activate sizable long-distance electrodynamic interactions between resonating molecules (*1, 2, 6, 7*). The new

theoretical prediction makes it worthwhile the effort to experimentally check whether out-of-equilibrium collective oscillations of biomolecules can be activated. Here, for a model protein, the BSA (Bovine Serum Albumin) protein in watery solution, we show that, when suitably driven in a stationary out-of-thermal equilibrium state, it displays a remarkable absorption feature around 0.314 THz, identified as a collective oscillation of the entire molecule. Two different and complementary THz near-field spectroscopic techniques have been used in order to enhance the sensitivity to oscillations of the molecules: a plasmonic rectenna, and a micro-wire near-field probe. The outcomes are in very good agreement with the theory. The present findings motivate also suitable experimental attempts at detecting long range electrodynamic forces between biomolecules.

Progress in terahertz technology has enabled to look at biological systems with terahertz radiation, that is, in an energy domain (a few meV) which is of the order of the activation energy of many biological processes. Among these, collective excitations driven by metabolic activity were hypothesised in the late '60s by H. Fröhlich to account for the huge speed of enzyme reactions and for the fast encounters of the cognate partners of biochemical reactions (*1, 2*). In fact, collective oscillations of biomolecules, by bringing about giant oscillating dipole moments, would result in resonant (thus selective) electrodynamic forces acting at a long distance (*1, 6, 8*). Fröhlich proposed a model where the activation mechanism of collective oscillations is a Bose-like condensation of the normal vibrational modes of a biomolecule. In this model a biomolecule is considered as an open system through which energy flows under the simultaneous action of an external supply and of dissipation due to radiative, dielectric, and viscous energy losses. The Bose-like condensation in the lowest vibrational mode is predicted to occur when the energy input rate exceeds some threshold value. However, the condensation

phenomenon originally surmised by Fröhlich has been criticized and marginalized because its quantum formulation can be hardly maintained to be a realistic one for biomolecules. In fact, the frequency of collective oscillations is expected in the sub THz domain, around 10^{11} Hz, so that at room temperature $k_B T/\hbar\omega \gg 1$ which rules out a quantum description. Of course this does not apply to molecular orbitals and light induced electronic transitions which are always of quantum nature. Accordingly, we have worked out a classical version of the original Fröhlich model, finding that - remarkably - also in a classical context a Fröhlich-like phonon condensation phenomenon is possible (see Supplementary Material (SM)). This is illustrated in Figure 1 which, for an idealized macromolecule, displays the deviation from energy equipartition among the normal vibrational modes - computed by means of our classical version of the quantum Fröhlich model - as a function of the input energy amplitude a. When this parameter exceeds a threshold value, the lowest frequency mode deviates from equipartition by enhancing its strength at the expenses of the other normal modes. Though still representing a biomolecule in a very idealized way, this model predicts a classical condensation phenomenon worth an experimental effort to detect it.

Even though experimental evidence of the existence of collective modes of vibration of biomolecules has been provided *at thermal equilibrium* by means of Raman spectroscopy (*9*) already many years ago, and is still being the object of many investigations (*5, 10–14*), no experimental evidence was hitherto available of the possibility of exciting out-of-thermal-equilibrium collective oscillations of a biomolecule. Unveiling whether these can be activated amounts to understanding whether a necessary condition to activate long-range intermolecular electrodynamic forces (*7*) can be fulfilled. This is what motivated conceiving and performing the two biophysics experiments on which we report in the present work.

For both experiments a model protein has been chosen: the BSA (Bovine Serum Albumine) protein. This is mainly made out of α-helices, and is a ”model” since it is largely studied in the

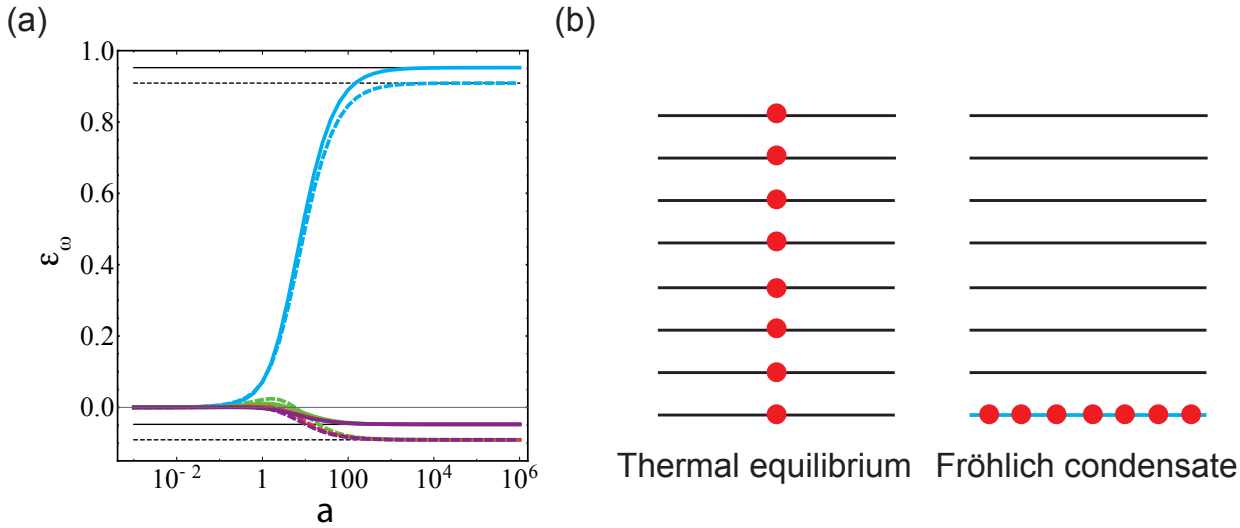

Figure 1: Classical Fröhlich-like condensation. At thermal equilibrium all the vibrational modes of a macromolecule fulfil energy equipartition. In the left panel this corresponds to $\epsilon_{\omega_i} = 0$. Non vanishing values of ϵ_{ω_i} (given in arbitrary units) refer to deviations from energy equipartition driven by increasing values of the injection energy amplitude denoted by the parameter a in abscissas (in arbitrary units). When the condensation sets in all the energy is concentrated in the lowest frequency vibrational mode. The right panel pictorially represents the two phases. The dotted and continuous lines refer to different spectral densities (see SM). The blue lines correspond to the lowest frequency mode $\omega = 1$. Only the a dependence of the five lowest modes is displayed.

biophysical chemistry literature. Our strategy to create a stationary out-of-thermal-equilibrium state of this molecule is to induce it by means of optical pumping, but without involving any optical transition of the protein, but rather by exciting some fluorochromes bound to each protein molecule. The optical excitation of these fluorochromes creates on each protein some "hot points" acting as the epicentres of a so-called "proteinquake" (*16, 17*) - better discussed in the following - and resulting in an energy transfer to the vibrational part of the protein. We used the Alexa488 fluorochrome which is covalently bonded at the lysine residues of the BSA and which is excited by means of an Argon laser (wavelength 488 nm). Some 0.19 eV per fluorochrome and per incident photon (the average energy difference between the absorbed and re-emitted photons) is thus available for an energy transfer to the protein and, partly, also to its

environment. By attaching an average number of 5 fluorochromes per protein a considerable amount of energy ($\gg k_B T$) can be continuously pumped into each protein. Two THz-near-field absorption spectroscopy setups of watery solutions of the protein (at 1 mg/mL concentration) operating into two distinct laboratories, have been used at room temperature (Fig. 2 (a), (c)). In both experiments, THz radiation is produced by tunable, highly-spectrally-resolved (< 300 Hz) and continuous-wave sources with an average power of 1 mW allowing an accurate detection of possible resonances. A typical experiment consists in three phases, during all of them the watery solution of proteins is illuminated with THz radiation performing a sweep in frequency, and thus allowing to measure the frequency dependence of the absorbed electromagnetic power (detected by the near-field probes) in the solution. During the first phase no extra illumination with the Argon laser is done; during the second phase the Argon laser is switched on to excite the fluorochromes bound to the proteins; finally, during the third phase the laser is switched off to check whether some memory and irreversible photochemistry effect (photobleaching) or sample heating are present. The use of near-field coupling of metal probes to the sample eliminates the Fabry-Perot interferences often seen in optical spectra taken in fluidic cells (*18*).

The first setup (Fig. 2 (a), (b)) used a micro-coaxial near-field probe put inside a metallic rectangular waveguide enabling a modal transition from TM_{01} Sommerfeld's to TE_{01} waveguide mode. The sub-wavelength diameter of the wire (12 μm) allows an extremely localized detection of the longitudinal component of the electric field at its end and on a volume of about 4 pL. The spectra were subtracted from the spectrum of pure water in order to remove artifacts coming either from the water absorption or from the geometry of the experimental setup (see SM). The second setup (*19*) (Fig. 2 (c), (d)) used a near-field probe rectenna composed of a planar metal bow-tie antenna with dimensions close to half-a-wavelength (at 0.3 THz) that enhanced the THz field in the feed gap region (volume of about 0.2 pL) and a plasma-wave field-effect transistor (FET) integrated in the feed gap of the antenna. When illuminated by THz

radiation, the antenna-coupled FET device provides a DC-voltage – between Source and Drain contacts – proportional to the THz-field intensity (*20–22*). A hemispherical silicon lens pressed on the back of the semiconductor substrate focused the THz radiation on the antenna simultaneously eliminating Fabry-Perot interference in the substrate (*23*). The spectrum of the protein solution obtained in the absence of blue light illumination was subtracted from the spectrum obtained with blue light illumination.

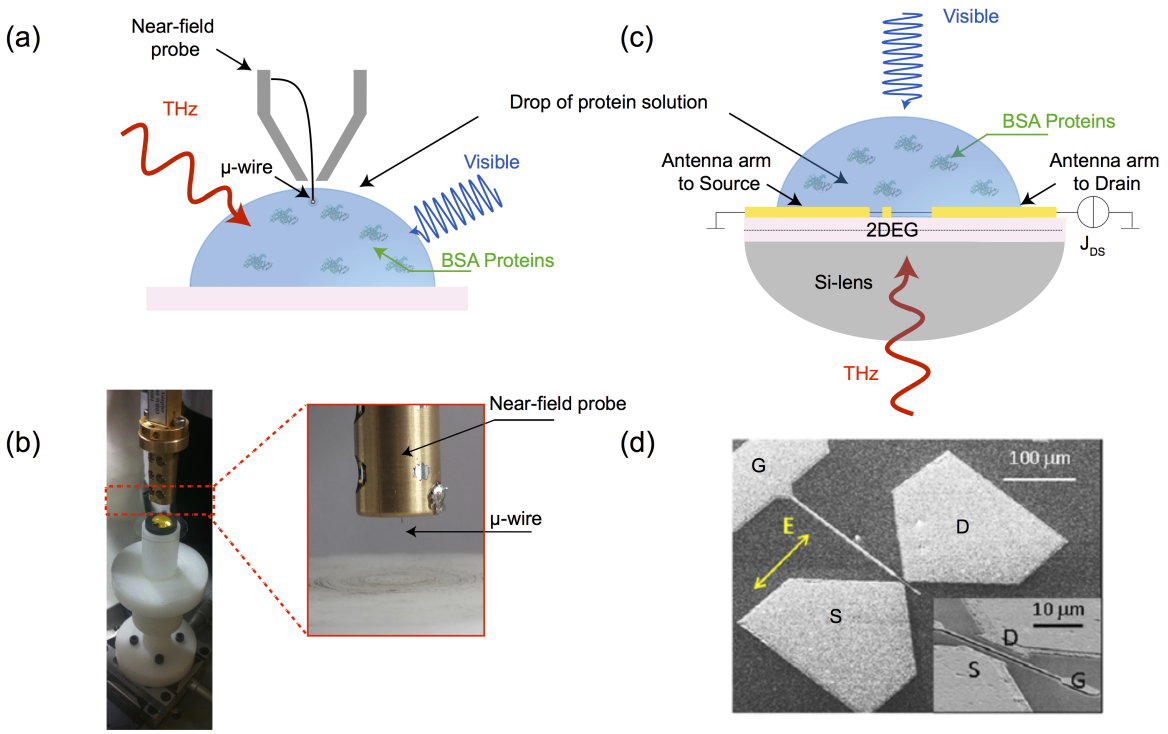

Figure 2: Experimental setups of THz absorption near-field spectroscopy. (a) A drop of the biological sample is placed under the near-field probe which is directly immersed inside the solution. (b) Picture of the near-field probe and its micro-wire. (c) A drop of the biological sample is placed above the near-field rectenna. (d) Electron-beam microscopy picture of the bow-tie antenna with its integrated FET.

Figure 3 (a) presents the spectra obtained using the micro-coaxial probe in the absence of blue light illumination (black circles) and in the presence of blue light illuminations of different durations (from 3 to 9 min). In the former case (no illumination), there was no specific spectral

feature in the studied frequency range while in the latter case (with illumination) we observed spectral resonances which become more evident for increasing duration of illumination. In particular, the strongest resonance appeared at 0.314 THz accompanied by two other minor resonances situated at 0.278 and 0.285 THz; these values did not depend on the time of illumination and the strength of the resonances saturated after 9 min of illumination. These results have full reproducibility. Figure 3 (b) presents the spectra obtained using the rectenna probe for two durations of blue light illumination. The spectra with illumination-on versus illumination-off were taken several times (two of them reported to show reproducibility). Also in this case we observed the appearance of evident resonances whose strength saturated at increasing durations of illumination (10 and 15 minutes in the two experimental runs reported). Long excitation times are needed because, under our experimental conditions, the energy dissipation rate and the energy supply rate are almost equal (see SM), so that a long time is needed to accumulate enough energy into each protein in order to make intramolecular nonlinear interaction terms sufficiently strong to activate the condensation phenomenon.

The spectra obtained using the two previously described methods and for the longer durations of illumination are compared in Fig. 3 (c). The principal resonance at 0.314 THz is perfectly reproduced using two completely different and complementary setups. Since THz extinction in water is huge (2000 dB/cm), the emergence of this spectral feature of the protein out of the water absorption background must be associated with the activation of a giant dipole moment. And this can happen only as a consequence of the activation of a coherent oscillation of the whole molecule, possibly together with a dipole moment strengthening due to an electret-like structuring of water dipoles of the first hydration layers of the proteins as hydration shell might contribute to the magnitude of the protein dipole moment (*24*).

Since the BSA is a heart-shaped globular protein, a possible lowest frequency mode is of the hinge-bending kind, what suggests to get a first rough estimate of the frequency of a global

oscillatory mode by schematizing the molecule as composed of two masses m, equal to half the total protein mass, joined by a spring of elastic constant k given by $k = EA_0/l_0$, where E is the Young modulus of the protein, A_0 and l_0 are its transverse section and length at rest, respectively. Using $m = 33$ kD, $A_0 \simeq 1.2 \times 10^{-13}$ cm^2, $l_0 \simeq 1.2 \times 10^{-7}$ cm, and $E = 6.75$ GPa, we find $\nu = (1/2\pi)\sqrt{k/m} \simeq 0.300$ THz which is close to the main resonance observed at 0.314 THz. Since the BSA molecule can be modeled to first order as a three-dimensional elastic nanoparticle (*13*), a more refined approximation is obtained by modeling the protein with an elastic sphere and then considering its vibrational frequencies.

The fundamental frequency of a spheroidal deformation mode of an elastic sphere is given by the formula (*25*)

$$\nu_0 = (1/2\pi)[2(2l+1)(l-1)]^{1/2} \left(\frac{E}{\rho R_H^2}\right)^{1/2} \tag{1}$$

which holds for $l \geq 2$. Using the following data for the BSA protein: Young modulus $E = 6.75$ GPa obtained at room temperature using Brillouin light scattering of hydrated BSA proteins (*26*), hydrodynamic (Stokes) radius $R_H = 35$Å, and specific volume $1/\rho = 0.74$ derived from small-angle X-ray scattering (SAXS) experiments (*27*), we find for the lowest mode ($l = 2$) the frequency $\nu_0 = 0.308\ THz$ which agrees within an error of about 2% with the observed peak value at $\nu = 0.314\ THz$. Though such a modeling is unrealistic in what it does not take into account the details of the protein structure and the associated normal modes (*28*), it nonetheless catches a relevant aspect of the global deformation dynamics of the BSA molecule, namely the activation of a collective oscillation, also suggesting that the physical parameters adopted correspond quite well to the situation investigated. Secondary resonances are also present in both spectra. A possible explanation could be tentatively given considering torsional modes. These could be activated at the frequencies given by the relation (*25*)

$$\nu_t = \nu_0 \left(\frac{(2l+3)}{2(2l+1)}\right)^{1/2}, \qquad l \geq 2$$

where ν_0 is given by equation (1), whence, for $l = 2$ and $l = 3$, one finds $\nu = 0.257\ THz$ and $\nu = 0.246\ THz$ respectively. These could be associated with the two weaker absorption lines observed at $\nu = 0.278\ THz$ and $\nu = 0.285\ THz$. Here the larger discrepancy can be attributed to the non-spherical shape of the BSA, what entails the existence of different moments of inertia according to the rotation axis, whereas the breathing mode is insensitive to this fact. Minor peaks are observed at higher frequencies with the rectenna (falling outside the accessible frequency range of the near-field probe) when the protein solution is illuminated with blue laser light. However, the blue light illumination could produce spurious signals from the 2D electron gas of the FET junction as a consequence of electron-hole pairs excitation causing a change of the transistor channel conductivity. This effect is well known and studied in the literature (*29*) so that minor peaks could be instrumental artifacts due to this electron-hole pair creation effect. Let us stress an important point: computational normal mode analysis for proteins has shown nearly continuous vibrational density of states (*28*) which have also been proved nearly uniformly optically active. Moreover, the coupling of these vibrational modes with water results in broad absorption features (*30, 31*). But this is true at *thermal equilibrium*, whereas under out-of-equilibrium phonon condensation the energy content of all the normal modes is strongly depleted with the exception of the collective mode. Whence a narrow absorption feature. And, in fact, the computation of the function $L(\omega)$ in SM shows how a dipole actively oscillating at a given frequency entails an absorption feature of shape similar to the experimentally observed one.

According to our classical version of the Fröhlich model, it is also expected that the appearance of a collective oscillation should exhibit a threshold-like behaviour when increasing the energy flowing through the protein. Actually, Figure 4 (a) presents a clear threshold in the intensity of the resonance peak at 0.314 THz when the optical input power exceeds 10 μW. By using a classical formalism for the analysis of the out-of-equilibrium phonon condensation (see

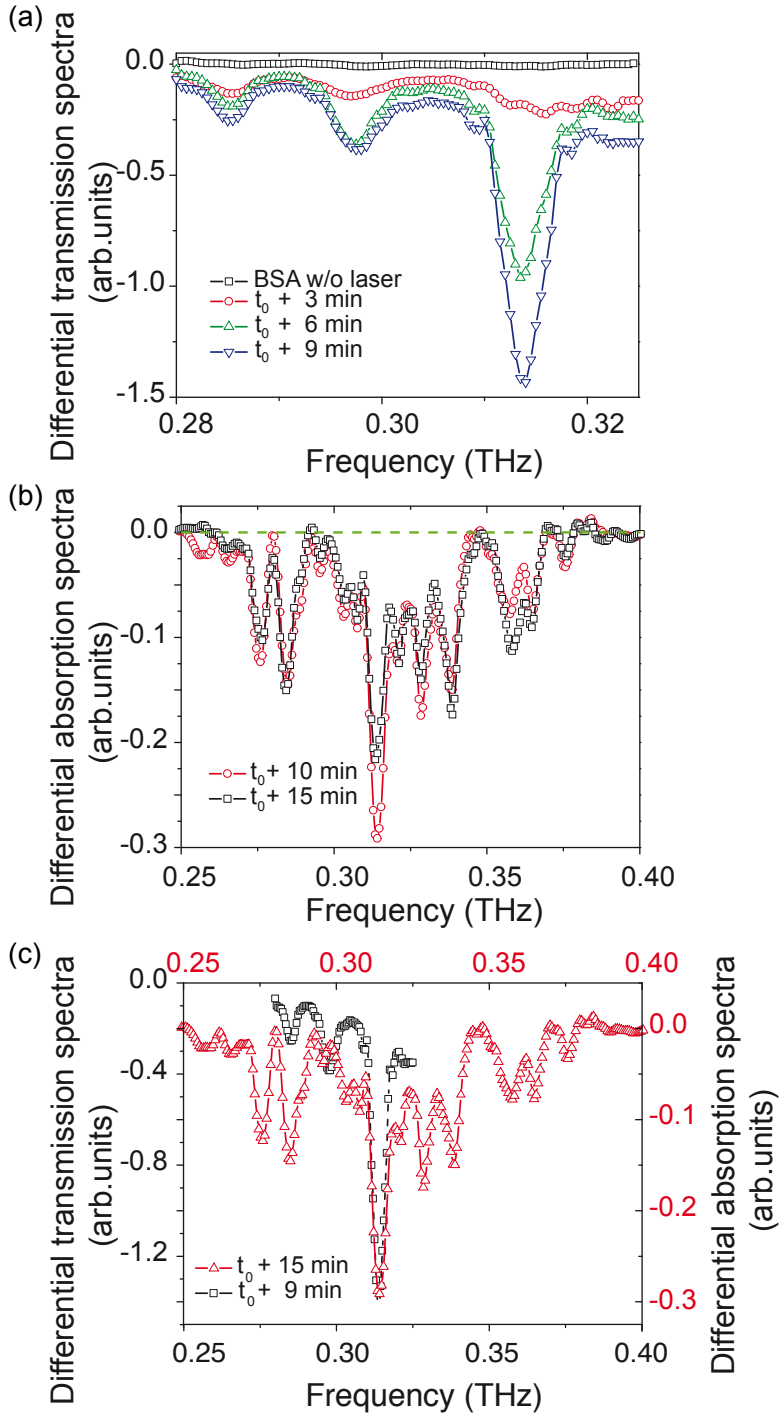

Figure 3: Differential transmission and absorption spectra as functions of the frequency. (a) Spectra obtained using the microwire probe after subtraction of the water spectrum in the absence of illumination and in the presence of illuminations for the reported durations. (b) Spectra obtained using the rectenna, after subtraction of the protein solution without illumination, for the reported durations. (c) Comparison of the two normalized spectra for the longest illumination durations.

SM) we have calculated the intensity of the normal vibrational modes of the BSA-protein as a function of the source power injected through the protein. Figure 4 (b) highlights a threshold-like behaviour of the intensity of the fundamental mode that accumulates the energy at the expenses of the excited modes, in *qualitative* agreement with the experimental outcome. By increasing the number of modes included in the calculation this threshold becomes more and more evident. The experimental and theoretical results reported in Figure 4 agree also in displaying a saturation effect occurring at large values of the energy input rate.

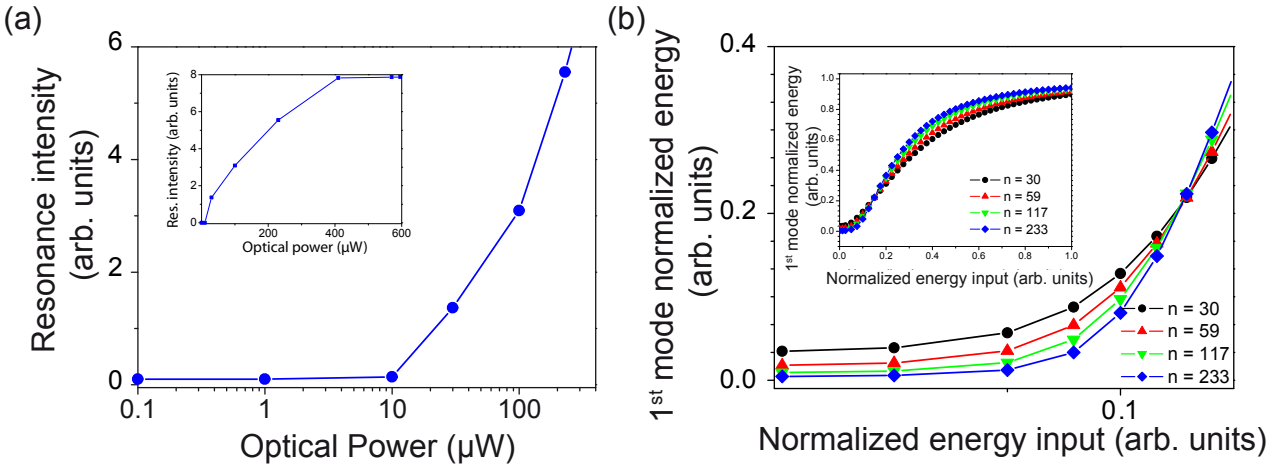

Figure 4: Threshold-like behaviour of giant dipolar oscillations. (a) Intensity of the resonant peak measured at 0.314 THz as a function of the optical laser power. (b) Normalized energy of the fundamental mode calculated as a function of the normalized source power. The different curves correspond to the reported numbers of normal modes of the BSA protein. Theory and experiment are in *qualitative* agreement.

The observed spectra are certainly due to the light-excited protein because the spectral feature at 0.314 THz was not observed by illuminating: *i)* water alone; *ii)* a watery solution of the fluorochrome in the absence of the protein; *iii)* a watery solution of the BSA protein without the bound fluorochrome (see SM). On the other hand, the observed spectral line at 0.314 THz immediately disappears by switching off the laser. Remarkably, the spectra obtained with two independent and different experimental setups, based on two different methods of detection of

the THz radiation, operated in two different laboratories, show a strikingly good overlap of the respective absorption line profiles at 0.314 THz. A result in excellent agreement with the frequency of 0.308 THz predicted for the spheroidal (collective) vibrational mode computed on the basis of recent experimental measurements of the relevant parameters of the BSA protein. This triple concordance among the results so far obtained should be enough to exclude experimental artifacts. This notwithstanding various sources of artifact for the observed phenomenology were considered. A first objection suggests itself, namely that the observed phenomenology is just a trivial heating effect due to the laser light. This would be true in the absence of a frequency dependent response to the injection of energy into the proteins. Heating indeed means increasing of the kinetic energy of the atoms and group of atoms of the protein entailing energy equipartition among the vibrational modes of the protein. Hence neither a frequency dependent effect nor a threshold effect for the energy input rate would have been measured, but just a new thermal equilibrium state would have been achieved. To the contrary, each protein - submitted to continuous energy feeding and energy dissipation - behaves as an open system undergoing a non-equilibrium phase transition: when the ratio between energy gain and losses exceeds a critical value a collective behavior sets in producing the phonon condensation (see SM). Then another question arises about the conversion mechanism of the visible light energy absorbed by the electrons of the complex protein/dyes into the vibrational modes of the proteins. This mechanism of rapid intramolecular dissipation of energy through quake-like structural motions as a consequence of a perturbation (such as the breaking of a chemical bond or the absorption of photons (through electronic transitions) is being given increasing experimental attention (*16, 17, 32, 33*) and is referred to as "protein quake". Similarly to an earthquake, this effect describes how a protein strain is released at a focus or "hot-point" (in our case the fluorochromes) and then rapidly spreads as a structural deformation through waves, thus exciting protein vibrational modes. Another source of artifact could be the apparition of standing waves and related

interferences that could have been easily identified. Moreover, there is no reason for such interferences to manifest themselves as a consequence of the blue light excitation of fluorochromes [SM Figure 6(b)].

Several terahertz spectroscopic studies have reported about collective modes of proteins, still performed at thermal equilibrium and mainly carried on using dry or low-hydrated powders because of the very strong absorption of water (*5, 10 –12*). More recent studies also addressed solvated proteins (*13, 14*). Other recent studies on solvated BSA in THz (*34*) and sub THz frequency range have shown (*24*) broad resonances due to an efficient coupling of low frequency modes of the protein with the surrounding water, but - again - all of these works are at *thermal equilibrium*. In common with these previous studies, our present work confirms the relevance of the coupling of the protein with the surrounding water molecules. In fact, the strong absorption feature that we observed in a watery solution of the BSA protein put *out of thermal equilibrium*, reveals that the protein vibrating in its collective mode has to be dressed by ordered layers of water molecules in order to attain an effective dipole moment sufficiently large to overcome the strong absorption of bulk water.

We anticipate that our result could open a broad domain of systematic investigations about out-of-equilibrium activation mechanisms and properties of collective oscillations of different kinds of biomolecules. Furthermore, as our result explains why electrodynamic interactions between biomolecules have hitherto eluded detection, it motivates new efforts to detect them (*35*).

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Supplementary materials

Materials and Methods

Supplementary Text

Figs. S1 to S4

References *(36-57)*